



Research article

Nitrate improves hackberry seedling growth under cadmium application

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ARTICLE INFO

Keywords:

Plant biology
Environmental science
Natural hazards
Ecology
Biological science
Biochemistry
Food safety
Heavy metal
Landscape
Nitrogen
Ornamental plants
Photosynthesis
Plant tolerance
Proline
Transpiration

ABSTRACT

The environmental toxicity of heavy metals in particular cadmium is a public concern. Cadmium is toxic for all living organisms including plants; however, plant species may show different tolerance to the presence of cadmium in their root medium. Adopting practical strategies may reduce cadmium bioavailability or increase the plant tolerance. In the present study, interaction of nitrate was investigated on cadmium treatment in hackberry (*Celtis australis* L.) seedlings. Different levels of nitrate (0, 50 and 100 mg/L) and cadmium (0 and 5 mg/L) were applied to seedlings via irrigation water during two consequence years. The treatments were arranged in a factorial with completely randomized design in four replications. The results of ANOVA showed that the cadmium-nitrate interaction was significant on leaf Cd concentration and root dry weight at $P = 0.01$, and on carotenoids and leaf dry weight at $P = 0.05$, while it was not significant on the rest of traits. Application of cadmium had no significant effect on new shoot growth, leaf chlorophyll and leaf fresh weight; however, it significantly reduced stomatal water conductance and photosynthesis rate, while it increased leaf transpiration rate, root and stem fresh weights, leaf Cd and proline concentrations. Application of nitrate levels, on the other hand, constantly increased the leaf nitrate concentration, new shoot growth, leaf fresh and dry weights, root fresh weight, stomatal water conductance and photosynthesis rate, whereas it reduced the necrotic points of leaves. The results indicated that the growth characteristics of hackberry seedlings were mainly influenced by nitrate but not cadmium application, and this ornamental tree is a tolerant species to high soil Cd levels.

1. Introduction

Heavy metals have been a component of our environment in a balanced level without significant problem; however, human activities toward modern life and industrialization have increased the heavy metal distribution and bioavailability (Lamastra et al., 2018; Vysloužilová et al., 2003). Industrial wastes and wastewater application, metalliferous mining and smelting, energy and fuel production, vehicle emissions and agricultural fertilizer applications are among the most important human activities leading to enhanced heavy metal pollution of our environment (Khan et al., 2018; Souri et al., 2018). High pollution with heavy metals in urban areas particularly around metropolitan cities have frequently been reported (Khan et al., 2018; Lamastra et al., 2018). It has been shown that the concentrations of Cr, Ni, Cu, Pb, Zn, As, Hg and Cd are higher than their background values of soil in many urban areas and agricultural soils (Bose and Bhattacharyya, 2008; Vysloužilová et al.,

2003; Souri et al., 2018). In most cases, polluted sites are urban areas and often associated with agricultural practices particularly fresh vegetable production that various pollutants simply enter the human food chain (Ng et al., 2018; Souri et al., 2018).

Instead, ornamentals (in nursery or in landscape plantations) can be used in urban areas, and to be irrigated with refined wastewater containing degrees of heavy metals without significant problem (Wang et al., 2012; Hatamian et al., 2018). In addition, ornamentals particularly trees represent suitable plant species for soil remediation of heavy metals (Vysloužilová et al., 2003; Hatamian et al., 2018). Many perennial species particularly woody trees are good biomonitors of heavy metals (Yang et al., 2018). It has been shown that woody deciduous tree of common Ash (*Fraxinus excelsior*) is a useful biomonitor of many heavy metals such as Pb, Cd, Cu, Zn, Ni and Cr (Aksoy and Demirezen, 2006). Significant differences in the uptake of metals have been observed in willow varieties and clones (Yang et al., 2018; Vysloužilová et al., 2003).

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Received 9 August 2019; Received in revised form 27 September 2019; Accepted 14 January 2020

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Cadmium by far is one of the most toxic heavy metals that its availability and biological concentrations have been increased in recent decades due to different human activities (Ghosh and Roy, 2019; Lux et al., 2011). Chronic exposure to Cd can have adverse effects such as lung cancer, pulmonary adenocarcinomas, prostatic proliferative lesions, bone fractures, kidney dysfunction, and hypertension (Jaishankar et al., 2014). In plants, cadmium can have different toxicity effects on metabolic processes including photosynthesis, respiration, tissue water relations and the uptake of nutrient elements (Ghosh and Roy, 2019; Lux et al., 2011). Despite plant species generally differ in their tolerance to heavy metals and cadmium pollution; however, different strategies have been suggested and applied to reduce heavy metal's bioavailability or to increase plant tolerance to their exposure. Foliar or soil application of some beneficial elements such as silica, potassium, calcium, mycorrhiza and nitrogen forms have been reported to influence plant heavy metal tolerance (Rady et al., 2019; Guo et al., 2019; Konate et al., 2017; Shi et al., 2019). Nitrogen is a mineral macronutrient with profound effects on plant metabolism (Marschner, 2011). The form, the time and the application rate of nitrogen and nitrogenous compounds may significantly influence many morphophysiological characteristics of plants. Nitrate is a major N form for plant uptake with different roles in plant metabolism that can significantly influence the plant tolerance to environmental stresses including cadmium toxicity (Marschner, 2011; Souri and Hatamian, 2019; Rady et al., 2019).

Hackberry is a relatively tolerant ornamental tree to many environmental stresses including water shortage, representing it as a suitable candidate for landscaping in arid regions. On the other hand, nitrogen and especially nitrate application may have influence on plant growth under stress conditions. Therefore, in the present study, the growth performance of hackberry seedlings was evaluated under interaction of nitrate and cadmium applied via irrigation water during two consecutive growing seasons.

2. Materials and methods

2.1. Experimental conditions

This experiment was conducted using black plastic pots and under protected plastic greenhouse during two consecutive years of 2016 and 2017. The pots with 12 L volume were filled with 10 kg air dried soil. The analysis of soil showed that the soil had a relatively moderate level of fertility with silty-loam texture, EC of 0.415 dS/m, pH of 7.17, 2.45% organic carbon, 78 mg/kg N, 15.2 mg/kg P and 256 mg/kg K. In addition, a soluble NPK + Mg + trace nutrients was also added and mixed thoroughly at a rate of 150 mg kg⁻¹ soil. One-year-old hackberry (*Celtis australis* L.) seedlings were purchased from a local nursery and transplanted to the pots on the first of April 2016. Seedlings were irrigated with just tap water for three weeks after transplantation. Thereafter, different levels of nitrate and cadmium and their combination treatments were applied to seedlings via irrigation water, and for two consecutive growing seasons during 2016 and 2017.

2.2. Treatments application

Different levels of nitrate as factor A (a1:0, a2:50, a3:100 mg/L) and cadmium as factor B (b1:0, b2:5 mg/L) were applied via irrigation water in a factorial design with completely randomized arrangement and four replications. Each pot containing one seedling represented one replication. The treatments were a1b1 (zero nitrate and cadmium as control), a1b2 (zero nitrate and 5 mg/L cadmium), a2b1 (50 mg/L nitrate and zero cadmium), a2b2 (50 mg/L nitrate and 5 mg/L cadmium), a3b1 (100 mg/L nitrate and zero cadmium) and a3b2 (100 mg/L nitrate and 5 mg/L cadmium). The treatments were applied in a relatively long term program and during two years from April to November. Plants were irrigated (or received treatments) 2–3 times per week (depending on season), and based on a water content of 80–70 % of soil field capacity. Cadmium was

supplied as CdCl₂, and nitrate was supplied in the form of calcium nitrate (Ca(NO₃)₂).

2.3. Measurements

Plants were harvested (November 2017) after two years irrigated with water containing nitrate and cadmium combination treatments. Different growth characteristics of seedlings were recorded. For determination of leaf fresh and dry weights, 40 leaves were randomly selected from middle part of plant and their fresh weight were recorded using a digital scale. The leaves were dried in an oven for 48 h at 65 °C, and then their corresponding dry weight was recorded. At harvest, plants were cut in stems and roots and after gentle washing, their fresh weight and dry weight (oven dried for 72 h at 65 °C) were determined with a digital scale. The average leaf necrotic points were counted, and the new shoot growth was measured using a tape. Leaf transpiration rates, stomatal water conductance and leaf photosynthesis rates were determined using Li Cor (Model Li-6100, Li Cor Co., Lincoln, NE, USA), at full light conditions and at 11 o'clock in the morning from a well-developed leaf in the middle part of a new shoot growth.

The leaf concentration of chlorophyll (chl a, chl b and total chlorophyll) and carotenoids were determined following Yang et al. (2018). The extraction of fresh leaves was performed in DMSO until all pigments were taken out. The absorbance of extracts was determined by a spectrophotometer (Jenway 6305 UV/Visible) at 663 nm, 645 nm and 470 nm for chlorophyll and carotenoids measurement respectively. The chlorophyll content was calculated as mg/g fresh weight.

Nitrate was determined potentiometrically as described by Schouwenburg and Walinga (1975), in which 10 g of plant leaves were homogenized in 50 mL deionized water for 2 min with a pestle and mortar. The homogenates were transferred to a 100 mL centrifuge tube, and after centrifugation, the supernatant was used for determination of nitrate concentration using flow-injection-analysis after Cd-catalyzed reduction to N₂O. The leaf proline concentration was determined using 2 mL of the alcoholic extract, 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. The absorbance of the samples was then measured against different standard proline concentrations of 0, 5, 10, 20 and 40 mg/L and using spectrophotometer at 520 nm. The concentration of cadmium in leaf samples was determined by dry ashing at 550 °C for 6 h followed by nitric acid digestion and measurement by atomic absorption spectrometer.

2.4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the SAS Software. The averages were also tested for significance using the difference of least significant means (LSD) at 5% level. The tables and figures were prepared using Word and Excel Microsofts.

3. Results

The results of ANOVA (Table 1) showed that the interaction of cadmium-nitrate on root dry weight and leaf Cd concentration was significant at P = 0.01, and on leaf dry weight and leaf carotenoid concentration was significant at P = 0.05, and their interaction on the rest of traits was not significant. The simple effect of nitrate on leaf necrosis points, new shoot growth, leaf transpiration rate, stomatal water conductance, net photosynthesis rate, leaf chlorophyll b, total chlorophyll and carotenoids, and leaf concentration of proline, nitrate, Cd, leaf fresh and dry weights and root fresh weight was significant at P = 0.01, and on leaf chlorophyll a, stem fresh and dry weights and root dry weight was not significant (Table 1). The simple effect of cadmium on stomatal water conductance, leaf proline, leaf cadmium and root fresh weight was significant at P = 0.01, and on leaf necrosis points, new shoot growth, leaf transpiration rate, net photosynthesis and stem fresh weight was significant at P = 0.05, and on leaf concentrations of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, leaf nitrate concentration,

Table 1. Analysis of variances for different traits measured in the experiment.

Source of Variation	df	Leaf necrosis point	New shoot growth	Leaf net photos.	Leaf transpire.	Stomatal water conduct.	Leaf chl a	Leaf chl b	Leaf total chl	Leaf carotenoid
Nitrate(a)	2	22.5**	788.2**	1.15**	0.0002**	0.00082**	0.013 ns	0.078**	0.151**	0.371**
Cadmium (b)	1	4.01*	200.0*	0.55*	0.43*	0.00024**	0.00086ns	0.010ns	0.011ns	0.015ns
Nitrate*Cadmium	2	0.1ns	4.7ns	0.007ns	0.003ns	0.0000031ns	0.028ns	0.003ns	0.017ns	0.064*
error	54	1.12	35.65	0.84	0.06	0.000016	0.019	0.0054	0.02	0.015
CV		34.44	18.90	15.92	13.24	18.95	12.54	17.90	9.23	18.07
Source of Variation	df	Leaf FW	Leaf DW	Stem FW	Stem DW	Root FW	Root dry weight	Leaf proline	Leaf nitrate	Leaf Cd
Nitrate(a)	2	23.8**	6.5**	55.2ns	57.54ns	5954.2**	21.4ns	85.0**	384295**	10544.7**
Cadmium (c)	1	0.24ns	0.014ns	323.4*	151.67ns	13035.7**	106.3ns	30.7**	631.2ns	444091.8**
Nitrate*Cadmium	2	0.98ns	2.32*	117.8ns	74.59ns	97.43ns	215.3**	0.7ns	1413.1ns	10239.2**
error	54	1.66	0.540	76.36	41.08	1070.65	37.21	1.17	6351.75	460.56
CV		17.81	14.97	15.92	15.53	15.47	14.30	17.40	17.02	18.50

** , * and ns indicate significant difference at 1%, 5% and not significant difference, respectively.

leaf fresh and dry weights, and stem and root dry weight was not significant (Table 1).

The comparison of means at 5% level of LSD test showed that the sum of plant new shoot growth (Table 2) was not affected by cadmium; however, it significantly increased by increasing the application rate of nitrate in irrigation water. Leaf fresh weight was not significantly influenced by cadmium; however, there was a constant increase of this trait over application of nitrate levels up to 100 mg/L (Table 2). The interaction of nitrate and cadmium on leaf dry weight (Figure 1) showed that the significant highest and lowest leaf dry weight were observed in plants that received 100 mg/L nitrate (regardless of cadmium application), and in plants under no nitrate and cadmium application, respectively. At 0 nitrate level, application of Cd significantly increased leaf dry weight; whereas at 50 or 100 mg/L nitrate levels, application of 5 mg/L cadmium showed no significant difference compared to zero cadmium treated plants (Figure 1). Stem dry weight was not affected by cadmium or nitrate levels (Table 2); however, stem fresh weight was significantly increased by application of cadmium (but not nitrate) compared to no cadmium treatment. Application of nitrate and cadmium significantly increased root fresh weight (Table 2). In addition, the interaction of nitrate-cadmium was significant on root dry weight (Figure 2). The highest root dry weight was in a2b1 (50 mg/L nitrate and zero cadmium) and the lowest amount was in a1b1 (zero nitrate and zero cadmium) and a3b1 (100 mg/L nitrate and zero cadmium) treatments. At 0 and 100 mg/L nitrate, application of cadmium significantly increased root dry weight; however, at 50 mg/L nitrate, root dry weight was significantly reduced by cadmium application (Figure 2).

Application of 5 mg/L cadmium had no significant effect on leaf necrotic points, chl a, chl b, total chl and carotenoids concentrations (Table 3). However, significant changes in leaf necrotic points, chl b, total chl and carotenoids were observed under nitrate levels (Table 3). The highest number of necrotic points was observed at zero nitrate and

Table 2. Changes in new shoot growth, leaf, stem and root fresh or dry weight of hackberry seedlings under nitrate and cadmium treatment.

	New shoot growth (cm)	Leaf fresh weight (g)	Stem fresh weight (g)	Stem dry weight (g)	Root fresh weight (g)
Nitrate (mg/L)					
0	26.2c	6.2c	53.1a	39.5a	193.2b
50	31.0b	7.3b	55.9a	41.8a	220.7a
100	37.6a	8.2a	55.5a	42.4a	220.2a
Cadmium (mg/L)					
0	33.25a	7.4a	52.7b	39.8a	197.9b
5	29.91a	7.3a	56.9a	42.7a	224.8a

Nitrate and cadmium levels were applied via irrigation water during two consequence years.

Data are average of four replications and means comparison was done at 5% level of LSD test.

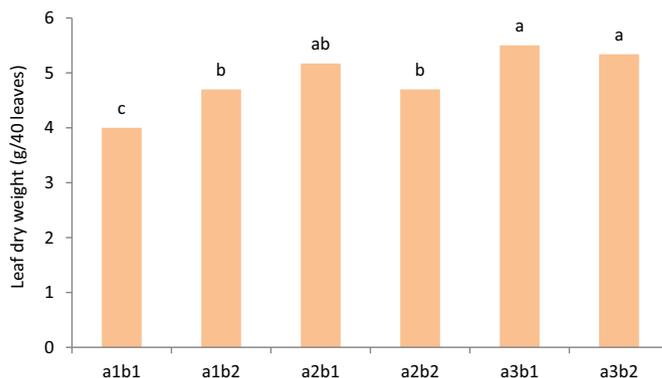


Figure 1. Changes in leaf dry weight under nitrate-cadmium interaction in hackberry seedlings. Nitrate (a1: 0, a2: 50, a3: 100 mg/L) and cadmium levels (b1:0, b2:5 mg/L) were applied via irrigation water during two consequence growing seasons. Data are average of four replications and means comparison was done at 5% level of LSD test.

the incident reduced with increasing nitrate application rate. Application of 100 mg/L nitrate resulted in significantly higher leaf chl b and total chl compared to plants received zero or 50 mg/L nitrate application (Table 3). The interaction of nitrate-cadmium on leaf carotenoids (Figure 3) showed that the highest leaf carotenoids were in a1b2 treatment and the lowest amount was in a2b1 and a2b2 treatments. At 0 level

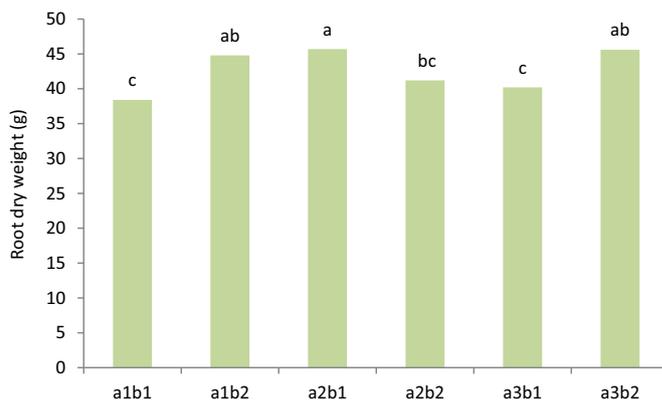


Figure 2. Changes in root dry weight under nitrate-cadmium interaction in hackberry seedlings. Nitrate (a1: 0, a2: 50, a3: 100 mg/L) and cadmium levels (b1:0, b2:5 mg/L) were applied via irrigation water during two consequence growing seasons. Data are average of four replications and means comparison was done at 5% level of LSD test.

Table 3. Leaf necrotic points and chlorophyll concentrations of hackberry seedlings under nitrate and cadmium treatment.

		No. of leaf necrotic points	Chl a (mg/g FW)	Chl b (mg/g FW)	Total chl (mg/g FW)
Nitrate (mg/L)	0	3.4a	1.14a	0.40b	1.52b
	50	2.2b	1.10a	0.36b	1.45b
	100	1.5c	1.14a	0.47a	1.61a
Cadmium (mg/L)	0	2.13a	1.12a	0.4a	1.519a
	5	2.61a	1.127a	0.42a	1.544a

Nitrate and cadmium levels were applied via irrigation water during two consequence years. Data are average of four replications and means comparison was done at 5% level of LSD test.

of nitrate, application of cadmium significantly increased leaf carotenoids; whereas at 50 mg/L nitrate there was no significant difference in leaf carotenoids between cadmium levels, and at 100 mg/L nitrate, there was an insignificant decrease in leaf carotenoids under 5 mg/L Cd application.

Application of 5 mg/L cadmium in irrigation water significantly increased leaf transpiration rate compared to those plants received no cadmium. Similarly, application of 100 mg/L nitrate resulted in significantly higher leaf transpiration than plants that received 0 or 50 mg/L nitrate (Table 4). Leaf stomatal water conductance and photosynthesis rate followed a similar trend, in which their amount was significantly reduced with application of 5 mg/L cadmium while they constantly increased with increasing the application rate of nitrate in irrigation water (Table 4).

The concentration of leaf proline was significantly increased under 5 mg/L Cd application in irrigation water (Table 4). There was also a constant increase in leaf proline by application of nitrate, as the significant highest leaf proline was in plants received 100 mg/L nitrate and then in those received 50 mg/L nitrate compared to 0 nitrate treatment (Table 4). Leaf nitrate concentration was not influenced by cadmium application; however, there was a constant increase in leaf nitrate concentration following increase the application rate of nitrate in irrigation water (Table 4).

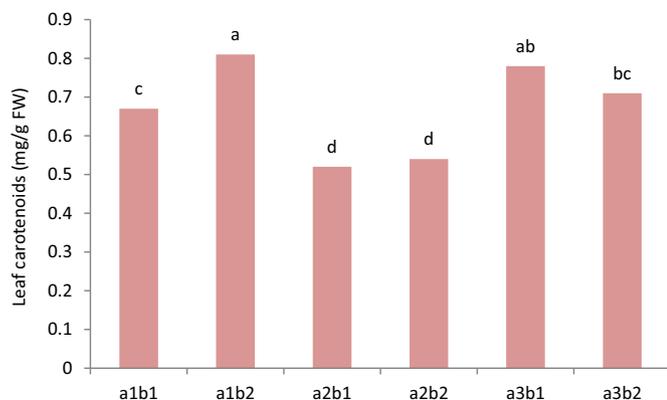


Figure 3. The interaction effects of nitrate (a1: 0, a2: 50, a3: 100 mg/L) and cadmium levels (b1:0, b2:5 mg/L) on leaf concentration of carotenoids. Data are average of four replications and means comparison was done at 5% level of LSD test.

Leaf Cd was significantly increased by Cd application, and nitrate-cadmium interactions showed significant effect on leaf Cd concentration. The increase in leaf Cd due to application of cadmium was different under nitrate levels, as application of 50 mg/L nitrate significantly reduced leaf Cd while application of 100 mg/L nitrate significantly increased leaf Cd compared to no nitrate treatment (Figure 4).

4. Discussion

In this study, application of 5 mg/L cadmium in irrigation water during two consequence growing season showed no significant toxicity effect on growth of hackberry seedlings. However, it significantly reduced stomatal water conductance and leaf photosynthesis rate, while significant increase in root and stem fresh weights, leaf Cd and proline concentrations, as well as leaf transpiration rate was observed following cadmium application. In our study, leaf Cd concentration was significantly increased by Cd application. Plant generally have high uptake rate of Cd following exposure or treatment with cadmium (Cheng et al., 2016; Arnamwong et al., 2015; Yang et al., 2018). In *Thlaspi caerulescens* shoot Cd concentration significantly increased from 67 to 555 mg/kg DW by cadmium treatment (Xie et al., 2009). Application of 5–25 μM Cd to different genotypes of willow trees significantly increased leaf Cd

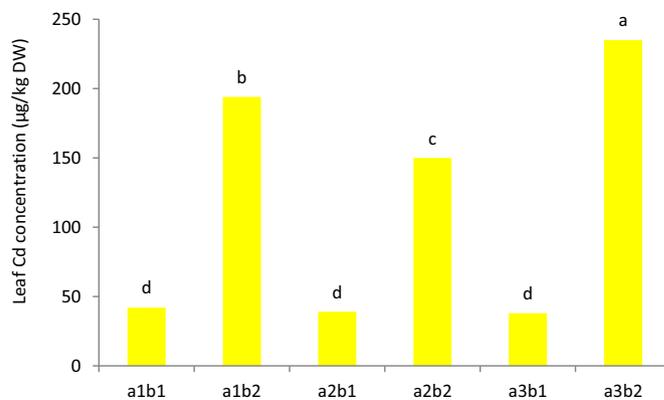


Figure 4. Leaf concentration of cadmium under different interactions of nitrate levels (a1: 0, a2: 50, a3: 100 mg/L) and cadmium levels (b1:0, b2:5 mg/L) applied via irrigation water during two consequence growing seasons. Data are average of four replications and means comparison was done at 5% level of LSD test.

Table 4. Changes in leaf transpiration rate, stomatal water conductance, photosynthesis rate, proline and nitrate concentrations under nitrate and cadmium treatment.

		Leaf transpiration ($\text{mmolH}_2\text{O m}^{-2}\text{s}^{-1}$)	Leaf stomatal conductance ($\text{molH}_2\text{O m}^{-2}\text{s}^{-1}$)	Leaf photosynthesis ($\mu\text{molCO}_2/\text{m}^2/\text{s}$)	Leaf Proline ($\mu\text{mol/gFW}$)	Leaf nitrate (mg/kg DW)
Nitrate (mg/L)	0	1.8b	0.151c	1.59c	4.35c	355c
	50	1.9b	0.021b	1.84b	6.23b	444b
	100	2.1a	0.027a	2.03a	8.11a	605a
Cadmium (mg/L)	0	1.85b	0.023a	1.91a	5.58b	471a
	5	2.01a	0.019b	1.73b	6.88a	465a

concentrations with a range of 165.0–1251.0 mg/kg leaf DW, and 22.9–331.2 mg/kg stem DW among the genotypes, despite Cd mainly accumulated in the roots (Yang et al., 2018).

No visible toxicity effect of Cd on hackberry seedlings could be due to low leaf concentration of Cd. Higher uptake rates and toxicity effects of Cd on plant growth have been reported in different studies (Yang et al., 2018; Wang et al., 2019). One or limited applications of cadmium generally has been shown to induce severe toxicity effects on different plant metabolic processes (Wang and Zhou, 2005; Li et al., 2009). Nevertheless, in the present study high amounts of Cd was applied via irrigation water during two growing years; however, the leaf Cd did not increase accordingly, resulting in no visible or severe toxicity effects of Cd on hackberry seedling growth. With willow seedlings, high contamination of heavy metals resulted in phytotoxic effects and caused chlorosis, partial defoliation and significant yield reduction of aboveground biomass in different willow clones (Vysloužilová et al., 2003). Similarly, severe phytotoxic symptoms in leaves and roots of ornamental willow genotypes including leaf chlorosis and root browning were noticed under 50 or 100 μM Cd treatment (Yang et al., 2018). In our study, without Cd application the leaf Cd concentration was below 50 $\mu\text{g}/\text{kg}$ DW and with high application rate of Cd during two growing season, the leaf Cd concentration was about 200 $\mu\text{g}/\text{kg}$ DW that seems still very low compared to many other plant species (Singh and Tewari, 2003; Xie et al., 2009; Yang et al., 2018). In addition, the bioavailability of Cd in pot soil was high enough (on average 17 mg/kg soil compared to 0.15 mg/kg soil in zero Cd application), indicating that soil Cd was not a limiting factor regarding low leaf Cd following cadmium application.

Such responses (low leaf Cd and no plant growth reduction) are rare among plant species. The growth characteristics of accumulator or hyper accumulator plant species have been also shown to reduce by high Cd levels (Verbruggen et al., 2009; Cheng et al., 2016; Shi et al., 2019; Liu et al., 2019). Our results showed that cadmium and nitrate increased root fresh and dry weights, while it is not the case in other studies. It has been shown that cadmium can significantly inhibit the root elongation of marigold (*Tagetes erecta*), scarlet sage (*Salvia splendens*) and sweet hibiscus (*Abelmoschus manihot*), and shoot elongation of marigold (Wang and Zhou, 2005) and many other plant species (White, 2012; Lux et al., 2011; Khan et al., 2018). About 30–40 % reduction in plant biomass was occurred in two Iris cultivars under Cd treatment (Han et al., 2007). In addition, in tomato it has been shown that in the presence of 35 μM CdCl₂ tolerant (Pusa Ruby) and sensitive (Calabash Rouge) genotypes exhibited a similar root growth reduction and trend of Cd accumulation in tissues. The dry matter of root and overall plants was reduced by Cd treatment (Borges et al., 2018).

Nevertheless, different tolerance or restriction mechanisms may be involved in low leaf Cd concentrations in hackberry seedlings in our study (White, 2012). Plant species may develop various mechanisms to counter the cadmium ions (Larsson and Asp, 2011; Wang et al., 2012). The root physiochemical characteristics are the main barrier to the entrance of Cd cations into plant tissues (Marschner, 2011). In this plant, root cells may actively avoid Cd cations due to an advanced uptake system of the roots (Cobbett and Goldsbrough, 2002; Marschner, 2011). In addition, roots of many plants can retain heavy metals and significantly reduce their translocation into the shoots (Bose and Bhattacharyya, 2008; Lux et al., 2011; Ghosh and Roy, 2019). The restriction in root to shoot transfer may be stronger for some specific heavy metals such as Pb and in particular Cd (Lux et al., 2011; Marschner, 2011) that offer a good opportunity regarding edible crops. The uptake and translocation of metal nutrients may severely get damaged under heavy metal application (Ghosh and Roy, 2019; Liu et al., 2019). Moreover, mineral elements particularly metals have generally negative interactions that significantly reduce their uptake by plant roots (Marschner, 2011).

Despite root exudates were not collected or analyzed in this study; however, hackberry root exudation may play an active role in chelation and precipitation of cadmium ions in the root medium. Changes in the amount and content of root exudation are intelligent responses of many

plant species to cope with soil stressful conditions (Souri and Hatamian, 2019). In addition, higher stem and root fresh weight as well as proline concentration induced by cadmium application represent adaptive tolerance mechanisms in the seedlings (Marschner, 2011; Ghosh and Roy, 2019). Increase in tissue's fresh weight is generally associated with changes in cell metabolism and increase in some cell protective osmolites such as soluble carbohydrates, organic nitrogen compounds (peptides, amides and amino acids) and some phytohormones (Poschenrieder and Barceló, 2004; Guo et al., 2019; Liu et al., 2019). Organic nitrogen compounds have unique role in sequestering and inactivation of toxic metals (Srivastava et al., 2004; Rady et al., 2019; Kato et al., 2019), and the role of phytochlatines has been well established in this regard (Cobbett and Goldsbrough, 2002; Marschner, 2011). It was shown that cadmium deposits extracellularly in some cell walls, and intracellularly on the inner surfaces of xylem vessels and some cytoplasm, in the root tip of two species of Iris L. treated with 1000 mg L⁻¹Cd in comparison to cells in control plants (Han et al., 2007).

In the present study, leaf transpiration, stomatal water conductance and photosynthetic rates (but not leaf greenness) were significantly reduced by application of 5 mg/L cadmium in irrigation water. Changes in leaf morphophysiological traits including greenness, stomata size and density, stomata hydraulic conductivity, transpiration and photosynthesis rates have been shown under heavy metal particularly cadmium treatment (Wang et al., 2019; Wang and Zhou, 2005; Yang et al., 2004; Shahid Umar and Iqbal, 2006; García-Sánchez et al., 2019). Reduction in hydraulic conductance of plant tissues is a common effect of heavy metals (Singh and Tewari, 2003; Poschenrieder and Barceló, 2004). Heavy metals probably via alteration in the amount of ethylene and ABA can change the stomata behavior and their opening (Ghosh and Roy, 2019). Cadmium can directly affect ion movement and water uptake of stomata cells (Singh and Tewari, 2003; Rucińska-Sobkowiak, 2016) and therefore results in prolonged stomatal closure in such a way that restrict carbon reception and accelerate water losing. Cadmium can also reduce the size and/or density of stomata on both sides of leaf surface and therefore change the balance of transpiration rate and carbon assimilation (Shahid Umar and Iqbal, 2006; Wang et al., 2019).

In the present study, application of nitrate more than cadmium influenced the growth of hackberry seedling. In addition, there was interaction of nitrate-cadmium on leaf Cd concentration, leaf and root dry weights, and leaf carotenoids. Application of 50 and particularly 100 mg/L nitrate improved seedling growth traits including: new shoot growth, leaf greenness, leaf fresh and dry weights, stem and root fresh weights, stomatal water conductance and leaf photosynthesis. Nitrogen is a key mineral nutrient in plant metabolism, as it constitutes 2–5% plant tissues dry weight (Marschner, 2011). Changes in soil physiochemical properties as well as changes in root growth characteristics probably are involved in the effects of nitrate on heavy metal uptake and accumulation in this study (Cheng et al., 2016). Nitrate is the most common form of nitrogen in plant nutrition, as application of reduced form of N can rapidly oxidized to nitrate by microbial activity (Souri and Hatamian, 2019). Many plant species have better growth and adaptability with nitrate rather than ammonium.

Different aspects of nitrogen application including N forms, N application rates and application time are effective on metabolic processes and plant growth responses (Marschner, 2011; Larsson and Asp, 2011). These aspects of nitrogen application are also important regarding the plants tolerance under environmental stresses and heavy metal treatments (Souri and Hatamian, 2019; Armamwong et al., 2015; Cheng et al., 2016; Xie et al., 2009). Nitrogen fertilization has been shown to improve Cd uptake by plants (Cheng et al., 2016; Xie et al., 2009). In *Thlaspi caerulescens* as a Cd hyper accumulator, supplying N as nitrate resulted in a doubling of Cd concentration in the shoots compared with the ammonium treatment, regardless whether solution pH was buffered or not (Xie et al., 2009). It has been suggested that the supply of ammonium rather than nitrate can favors Cd phytoextraction in some plant species (Cheng et al., 2016; Armamwong et al., 2015). Nevertheless, in *Panax notoginseng* it has been reported that nitrogen and potassium fertilization can significantly reduce cadmium

accumulation (Shi et al., 2019). Our results showed that increase or decrease in leaf Cd depends on the applied concentration of nitrogen (nitrate), as 50 mg/L nitrate decreased and 100 mg/L nitrate increased leaf Cd concentration. Morphophysiological responses to nitrogen fertilization always exist among plant species (Souri and Hatamian, 2019). In addition, nitrate application can significantly enhance the biosynthesis of a wide range of nitrogenous compounds effective on plant tolerance to stressful conditions (Marschner, 2011; Souri and Hatamian, 2019).

In this study, calcium nitrate was used as a nitrate source. Calcium as the companion cation of nitrate can have significant effect on Cd bioavailability, uptake, translocation and plant tolerance (Perfus-Barbeoch et al., 2002; Eeva and Lehtokoinen, 2004). Both nitrogen (nitrate) and calcium can induce higher cell membrane integrity under Cd treatment (Marschner, 2011; Souri and Hatamian, 2019). In addition, heavy metals can also enter the cells via different cationic channels (Perfus-Barbeoch et al., 2002), the main to be those of Ca and K uptake systems. In rice addition of calcium to the growth medium significantly reduced Pb root uptake (Kanu et al., 2019). It has been shown that cadmium with depolarization of Ca channels can enter the roots (White, 2012; Verbruggen et al., 2009). On the other hand, many minerals and organic compounds have been shown to reduce the adverse effects of heavy metals via foliar or soil application. Application of potassium fertilizer significantly reduced the tissues content of cadmium and increased plant cadmium tolerance in *Panax notoginseng* (Shi et al., 2019). In addition, the bioavailable Cd content in the soil decreased with the increasing of total potassium and available potassium in the soil (Shi et al., 2019). Similarly, foliar spray of S, P, and a mixture of both were effective to reduce the Cd concentration in rice grain (Liu et al., 2019).

In this study, application of 5 mg/L cadmium in a relatively long term via irrigation water showed no visible toxicity symptoms on hackberry growth except higher transpiration and lower photosynthesis rates. However, these adverse effects were not reflected in different tissue's biomass (leaves, stem and roots). In contrast, root and stem fresh weights were significantly increased over Cd application, indicating changes in efficiency of metabolic processes including photoassimilate allocation under cadmium treatment. The significant higher leaf dry weight was observed in those plants that received 100 mg/L nitrate, regardless of cadmium application compared to plants received no nitrate and cadmium. The concentration of leaf proline was increased by application of cadmium (indicating some degree of stress), and increasing nitrate levels further increased leaf proline concentrations probably to counter the negative effects of Cd cations. Application of cadmium showed no significant effect on leaf fresh weight; however, there was a constant increase in leaf fresh weight over application of nitrate levels up to 100 mg/L in irrigation water. Nevertheless, hackberry seedlings showed low uptake rate of cadmium, although high amount of Cd was applied in pot soil via irrigation water during two consequence growing seasons.

5. Conclusion

In the present study, the hackberry seedlings absorbed low concentration of Cd despite high amounts of Cd was applied into the soil via irrigation water. The growth characteristics of seedlings were more affected by nitrate rather than cadmium application. Our results indicate that hackberry is a very tolerant ornamental tree species to high soil Cd levels, probably via Cd uptake avoidance, and application of nitrate improved its growth under high Cd treatment. Moreover, hackberry represents a very suitable ornamental for landscape planning in polluted soils and urban areas.

Declarations

Author contribution statement

M. Hatamian: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

A. Rezaei Nejad: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

M. Kafi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

M. K. Souri: Conceived and designed the experiments; Analyzed and interpreted the data.

K. Shahbazi: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We put our especial thanks to Dr K. Bazargan for his gentle assistance and providing us with all laboratory requirements.

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